Original Articles

Effect of a single dose of diethylcarbamazine, albendazole or both on the clearance of *Wuchereria bancrofti* microfilariae and antigenaemia among microfilaria carriers: A randomized trial

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ABSTRACT

Background. Lymphatic filariasis is a major vector-borne parasitic disease. The global programme to eliminate lymphatic filariasis was launched in 1997 and currently over 570 million people are covered under it in 48 countries. Mass annual single-dose drug administration of diethylcarbamazine (DEC), co-administrated with albendazole for 5–6 years and mass distribution of diethylcarbamazine-fortified salt are the two strategies for elimination of filariasis.

Methods. Asymptomatic volunteers residing in Puducherry, India were screened for microfilaria (mf) by examining nocturnal thick blood smears. Those testing positive were randomly assigned to receive a single dose of DEC (6 mg/kg body weight) or albendazole 400 mg or both. Participants were hospitalized for 5 days. Membrane filtration count was used to assess microfilaraemia and ELISA (Og4C3) assay to measure circulating filarial antigens (CFA). Measurements were done before treatment and at 1, 2 and 3 years post-treatment. Viability of the adult worms was assessed by looking for the filarial dance sign (FDS) using ultrasound examination of the scrotum in men with hydrocele.

Results. Fifty-four microfilaraemic individuals were studied. The mf prevalence started decreasing only by day 180 post-treatment in the DEC group but much earlier in the other two groups (day 30 in the albendazole and day 90 in the DEC with albendazole group). The decrease in mf was marginal (17.6%, 26.3% and 27.8%, respectively) by the end of year 1 post-treatment, but significant (96.7%, 78.6% and 93.3%, respectively) by the end of year 2 post-treatment ($p < 0.05$). By the end of year 3, the level decreased to 80% in the DEC, 90% in the albendazole and to 100% in the DEC and albendazole groups. However, the mf intensity decreased significantly (by 39%; $p < 0.05$) by day 7 post-treatment in both the DEC and DEC with albendazole groups, but only by day 30 in the albendazole group. In all the drug groups, the prevalence as well as intensity of CFA returned to pre-treatment levels by the end of year 3 post-treatment.

Conclusion. Annual single-dose administration of all the 3 drug regimens significantly reduced antigenaemia levels. There were no significant differences in the efficacy and overall pattern of CFA clearance between the 3 drug regimens.

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INTRODUCTION

Lymphatic filariasis caused by *Wuchereria bancrofti* is a major public health problem in India. At present, efforts are under way to eliminate this problem and the mainstay of elimination programmes is single-dose, mass annual chemotherapy with a combination of diethylcarbamazine citrate (DEC) or ivermectin and albendazole.[1,2] It is necessary to assess and monitor the efficacy of such a regimen in terms of clearance of the parasite load and to decide on the duration and end-point of drug administration. It is important to establish end-point parameters to decide whether to stop or continue the mass drug administration programme (MDA).[3] Reports on the impact of the MDA programme have shown the utility of immunological markers.[4]

Several trials have been done to assess the efficacy of antifilarial drugs either individually or in combination with albendazole.[5–7] In most studies, parasite clearance was measured using conventional thick blood smear examination of samples collected at night. A few chemotherapy trials have employed highly sensitive immunotechniques, but assessed parasite clearance (antigenaemia) only at the end of the study period, which ranged from 1 month[8] to 2 years.[9] Also, since most of these studies were community based, incomplete coverage and poor compliance may have affected the efficacy of the drug. There are no systematic studies on parasite and antigenaemia clearance with different drug regimens. We did a hospital-based study on the impact of an annual single dose of DEC, albendazole or their combination on the adult worm burden, microfilaraemia and antigenaemia among *W. bancrofti* carriers. The findings at 1 year following drug

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administration have been reported earlier.[10] We present the results of the second and third year of follow up of the study.

METHODS

Selection of cases and drug administration

Blood samples were collected between 2000 and 2200 hours from healthy volunteers in Puducherry (formerly Pondicherry)—an area endemic for filariasis. Thick blood smears were examined for microfilariae (mf). Asymptomatic microfilaraemic individuals were recruited for the study. Participants were admitted to the Government General Hospital, Puducherry for a period of 5 days. They were randomly assigned to one of three single-dose drug regimens: (i) albendazole 400 mg (SmithKline Beecham, India); (ii) DEC 6 mg/kg of body weight (Wyeth Ltd, India); or (iii) both. All participants provided written, informed consent. The details of the study area, participants, treatment and follow up have been published previously.[10]

Collection of blood samples for microfilarial count and immunoassay

Venous blood samples (2 ml) were collected from mf carriers at different time points during the night. One ml blood was transferred to a heparinized tube for membrane filtration assay to determine the mf count and the rest was used for separation of serum. The heparinized venous blood samples were processed by membrane filtration[11] and mf on the membrane were stained as per standard procedures using JSB stain,[12] their numbers counted under a microscope and recorded. The separated sera were stored at –20 ^{o}C until the antigen assays were carried out.

Assay for circulating filarial antigens

Circulating antigens of *W. bancrofti* (CFA) present in the serum samples were detected using Og4C3 ELISA. Og4C3 ELISA was carried out as per the instructions of the manufacturer (JCU Tropical Biotechnology Private Limited, Queensland, Australia). The samples were run in duplicate and the optical density (OD) was read at 414 nm. The ODs of 7 standard antigen controls provided in the kit were used to construct the standard curve. The antigen titre was determined for all individuals using the mean OD values and the standard curve. Samples with an OD value of more than or equal to Standard No. 4 were considered positive as per the manufacturer's guidelines.

Assessment of filarial dance sign (FDS)

FDS was assessed in male mf carriers. Each individual was subjected to ultrasonographic examination for the FDS in nests of adult worms using HP SONO 100 colour Doppler (Philips, Holland). The probe had a linear, 7.5 MHz transducer and was set at real-time mode during examination. Both sides of the scrotum were examined serially, first applying the probe to the root of the scrotum and then to the area adjoining the epididymis and testes. Inguinal lymphatic vessels and lymph nodes and both the thighs were examined. Finally, the lymphatic vessels and nodes of axillae and upper arms were also examined. Each examination lasted 20 to 30 minutes.

Statistical analysis

Chi-square test was used to compare the positivity of mf and CFA between groups. For comparing the mean OD values between groups, the Kruskal–Wallis non-parametric test was used. To compare the means of two independent samples, Mann–Whitney U-test was used and to compare the means of related samples, Wilcoxon signed rank test was used. For all these tests, the statistical level of significance was considered to be $p<0.05$.

RESULTS

Fifty-four asymptomatic volunteers with mf, between 10 and 57 years of age (mean 24.7 years), were admitted to the Government General Hospital, Puducherry for 5 days and randomly allocated to one of three drug groups. The pre-treatment mf and CFA intensities of participants in each group are shown in Table I.

Impact on prevalence of CFA

The pre-treatment prevalence of CFA as assessed by Og4C3 assay was 88.2% in the DEC group, 94.7% in the albendazole group and 94.1% in the co-administration group (Fig. 1). The difference in prevalence was not statistically significant.

Following treatment, the prevalence of CFA in the DEC group decreased to 17.6% at the end of year 1, after which it increased to 93.8% by the end of year 2. By the end of the year 3, the level decreased to 80% in the DEC group; to 90% in the albendazole group and to the original level of 100% in the co-administration group. The prevalence of CFA was significantly reduced at 1 year post-treatment in the albendazole and co-administration groups; but by the end of years 2 and 3, the prevalence reached close to pre-treatment levels (Fig. 1).

Intensity of CFA

The pre-treatment mean intensity of CFA in the three groups was similar ($p=0.871$; Table I and Fig. 2). The intensity of CFA rose to pre-treatment levels in all the groups by the end of years 2 and 3.

Microfilaraemia

The mf positivity pattern was different from that of the CFA. It remained unaltered till day 180 post-treatment in the DEC group, till day 30 in the albendazole group and till day 90 in the co-administration group (Fig. 1). At the end of 1 year post-treatment, the mf prevalence decreased marginally to 82.4%, 73.7% and 72.2%, respectively. By the end of year 2, it fell to significantly low levels, i.e. 6.3%, 21.4% and 6.7% in the DEC, albendazole and co-administration groups, respectively. There was no significant difference in the intensity of mf between the 3 groups at the end of year 2 post-treatment. By the end of year 3, the mf

TABLE I. Pre-treatment level of microfilariae (mf) and circulating filarial antigens (CFA) in each drug group

Group	*n*	Microfilaraemia			Circulating filarial antigens		
		Mean (SD)	Median	Range	Mean (SD)	Median	Range
Diethylcarbamazine	17	133 (157)	52	22–542	0.39 (0.21)	0.46	0–0.64
Albendazole	19	129 (164)	56	22–606	0.49 (0.16)	0.52	0–0.71
Both	18	98 (57)	94	24–223	0.47 (0.18)	0.49	0–0.79

Arithmetic mean intensity of mf and CFA did not differ significantly ($p>0.05$) between the drug regimens

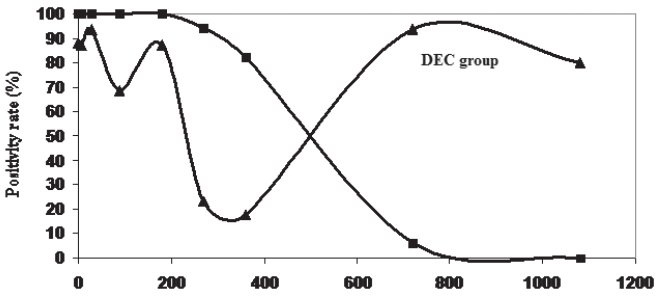

FIG 1a. Effect of a single dose of DEC on the prevalence of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

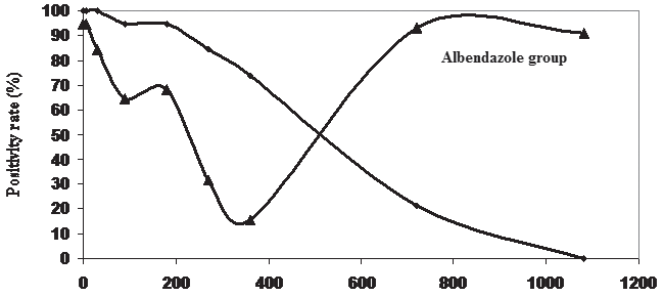

FIG 1b. Effect of a single dose of albendazole on the prevalence of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

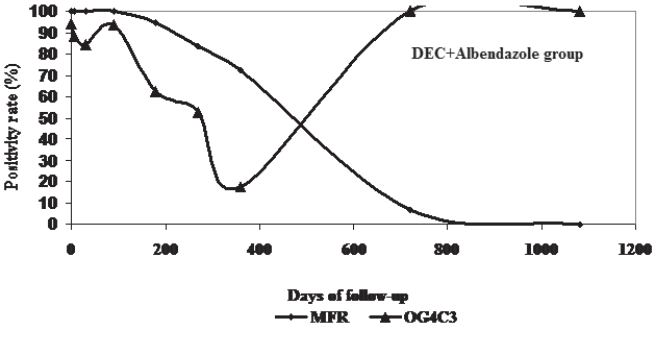

FIG 1c. Effect of a single dose of DEC and albendazole on the prevalence of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

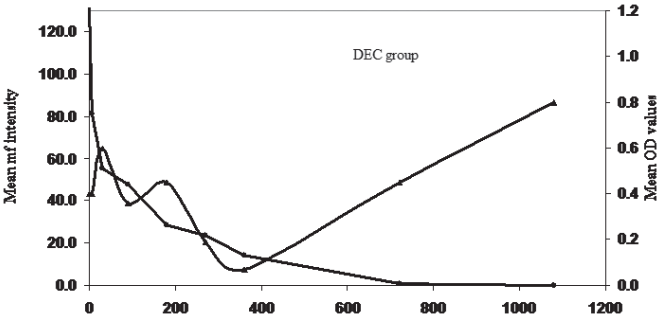

FIG 2a. Effect of a single dose of DEC on the intensity of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

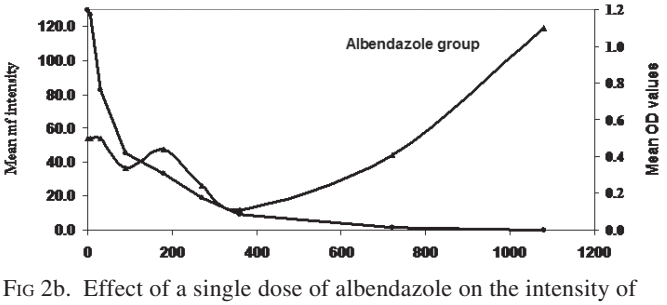

FIG 2b. Effect of a single dose of albendazole on the intensity of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

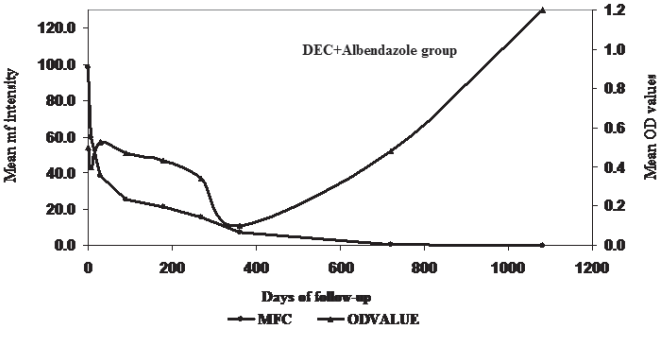

FIG 2c. Effect of a single dose of co-administration of DEC and albendazole on the intensity of antigenaemia and microfilaraemia among *Wuchereria bancrofti* mf carriers

prevalence decreased to zero in all the 3 groups. Unlike the CFA prevalence, which decreased to significantly low levels by the end of year 1 post-treatment and then increased almost to pre-treatment levels by the end of year 2, the mf prevalence continued to decrease from marginally decreased levels at year 1 to significantly low levels by the end of year 2 in all the 3 groups.

Filarial dance sign

There were 7, 9 and 10 male mf carriers in the DEC, albendazole and co-administration groups, respectively. One year post-drug administration, while none of the individuals treated with either albendazole or DEC demonstrated FDS, one of the patients given a combination of DEC and albendazole showed the presence of

TABLE II. Effect of various drugs on the filarial dance sign in male *Wuchereria bancrofti* microfilarial carriers

Group	Pre-treatment		1 year post-treatment		2 years post-treatment	
	Examined	Positive	Examined	Positive	Examined	Positive
Diethylcarbamazine	7	5	7	0	6	0
Albendazole	9	4	8	0	7	0
Both	10	5	10	1	10	1

FDS (Table II). The same observations were made at the end of year 2. FDS was not done at the end of year 3 due to practical difficulties.

DISCUSSION

Several antifilarial chemotherapy trials on the efficacy of DEC or ivermectin and their combination with albendazole have been conducted by other workers in different countries.[13] However, most of these trials did not assess albendazole alone and in the few studies which did, the efficacy of the drug regimens was measured using mf clearance, which does not indicate the clearance of adult worms. Albendazole has been reported to be macrofilaricidal but not microfilaricidal[14,15] and hence clearance of mf is not an appropriate parameter for assessing its efficacy. Its efficacy needs to be assessed using immunodiagnostics, which can detect the presence of not only mf but also developing and adult worms. Hence, detection of infection (antigen positivity) by means of antigen assay is also required in addition to detecting the infectivity (mf positivity). Bockarie *et al.*[16] reported a significant decrease in antigen prevalence at 24 months with DEC and albendazole compared with either drug alone.

An important finding of our study was that by the end of year 2, the antigen levels had returned to almost pre-treatment levels and continued to remain so after year 3 among treated individuals; but the mf intensity and prevalence remained low in all 3 groups. This indicates that at the end of year 1 post-treatment with any of the 3 drug regimens, as many as 30% of worms were still metabolically active, as reflected by the prevalence of antigenaemia at this time point. By the end of year 2, almost all adult parasites appeared to be reactivated. However, FDS was not observed among male mf carriers even 2 years after treatment. The reasons for such a pattern could be (i) re-infection in treated individuals who continued to reside in filariasis-endemic areas and where infection transmission would be sustained by the parasite reserve in the community as well as vectors, and/or (ii) reactivation of adult worms that were probably inactivated by the drug. The first reason appears more likely since even in the absence of FDS among patients with hydrocele, antigenaemia levels were high. This suggests the presence of enough new pre-adult stage (L3, L4 or L5) parasites as a result of re-infection, which caused antigenaemia similar to pre-treatment levels. However, it is not known whether nests in the scrotal region are formed when the parasite is in the L3, L4 or L5 stage and exhibits FDS.

The second possibility is supported by the fact that nematodes undergo a sort of quiescence such as hibernation or reversible suspended animation under the influence of environmental stress.[17] Embryos of the free-living nematode *Caenorhabditis elegans* have been reported to undergo an extreme form of quiescence during anoxia and the process is controlled by spindle checkpoint components such as SAN-1 or MDF-2.[18] A partial effect of albendazole on the adult parasites has also been reported based on ultrasound and antigen assays.[19–21]

Another striking difference in outcome between the different drug regimens is the duration of effectiveness. The reduction in CFA intensity as well as prevalence lasted up to 9 months in the DEC group and up to 1 year in the albendazole and co-administration (albendazole and DEC) groups. This was also reflected by the reduction in intensity and prevalence of mf. Re-treatment should ideally be done before antigens levels start rising, in order to be more effective. This means that DEC has to be administered at 9-monthly intervals, since antigen levels start rising by month 9 among individuals treated with this drug. The other 2 drug regimens can be administered annually since their efficacy lasted for a year, with antigen levels rising at the end of year 1 post-treatment.

Different batches of testing kits or cards were used for CFA measurements at different time points. However, the variations in OD values between the assays performed ranged from 0.057 to 0.128 between batches. Each plate error was corrected by appropriate blanking of the wells.

There was a difference in the initial reduction of mf intensity between the three groups. The mf intensity was drastically reduced by day 7 post-treatment in the DEC and combination groups, whereas in the albendazole group such a reduction was observed only after a month post-treatment. This could be due to the difference in the mechanism of action of the drugs used. DEC is reported to act as a microfilaricide by modulating the non-adoptive arm of the host immune mechanism[22–25] through interfering with the arachidonic acid metabolism of the filarial parasites and host[26] and hence kills immediately. On the other hand, albendazole is known to act by depolymerization of the tubulin protein present in the muscle cells of the parasite intestine, which would eventually lead to starvation followed by death of the parasite, as reported in intestinal parasites[27–29] and in *Brugia malayi*.[30]

We found that albendazole has significant antifilarial activity. A recent Cochrane review[5] indicated that there was insufficient evidence to confirm or refute the fact that albendazole co-administered with DEC or ivermectin is more effective than DEC or ivermectin alone in clearing mf or killing adult worms. In our study, all the 3 drug regimens were effective in reducing CFA and mf density and prevalence. However, the duration of effectiveness of DEC was about 9 months while that for albendazole and co-administration of the two drugs was about a year. It should be noted that re-infection is possible in an endemic area even after zero prevalence and absence of CFA. We found no strong evidence of death of adult parasites due to administration of an annual single dose. Our results, if confirmed by larger studies, would contribute to improving the national filariasis control programme.

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